[CONTRIBUTION FROM THE COLLEGE OF AGRICULTURE, TOHOKU IMPERIAL UNIVERSITY.]

INFLUENCE OF THE CHEMICAL STRUCTURE OF THE COMPOUNDS TO BE AMMONIFIED UPON THE RATE OF AMMONIFICATION.

BY K. MIYAKE.

Received July 12, 1917.

Since organic compounds have taken on a deeper significance in their relation to soil fertility, an investigation of the nature of soil organic matter has been made rather extensively by Schreiner and his colleagues.[1] Of the organic compounds thus far isolated from soils, some have beneficial action upon the growth of plants, while others are distinctly harmful.[2] It is also believed that organic substances which are toxic at higher plants in water culture lose completely or partially their toxicity when added to soil.[3] The loss of toxicity seems to be due to the disappearance of the toxic substances as being decomposed by biological means.[4] The rate of decomposition of a compound not only depends upon the kind of soil,[5] but also upon the chemical structure of the compound. Evidently some organic nitrogenous compounds readily ammonify, others do not.[6] While in some soils the compound is readily ammonified, it does not undergo similar process in other soils. The problem of toxicity of toxic organic matter in the view of practical agriculture is, therefore, closely associated with whether the compound in question will be decomposed readily in soils or not. For this very reason, the studies on the decomposition of various organic materials in soils are of great interest and importance in the field of soil fertility investigations, yet there is little evidence to show the relation between chemical structure of the compounds and their facility of decomposition in soils. Consequently, I have started my

[1] Schreiner, *et al.*, THIS JOURNAL, 30, 1295, 1599 (1908); 31, 116 (1909); 32, 1679, 1680 (1910); 33, 78, 255, 1412 (1911); 34, 99, 1242, 1260 (1912); *J. Ind. Eng. Chem.*, 7, 860 (1915); *J. Biol. Chem.*, 8, 381, 385 (1910); 9, 9 (1911); *J. Franklin Inst.*, 171, 295 (1911); 172, 145 (1911); *J. Agr. Res.*, 1, 357 (1913); 3, 175 (1914); 6, 1043 (1916); *Science*, 33, 340 (1912); 35, 390 (1912); 36, 577 (1912); U. S. Dept. Agr., Bur. Soils, *Bull.* 53 (1909); 74 (1910); 88 (1913); *8th Intern. Cong. Appl. Chem. Orig. Com.*, 15, 147, 247 (1912).

[2] Schreiner, *et al.*, U. S. Dept. Agr., Bur. Soils, *Bull.* 47 (1907); 70 (1901); 83 (1911); 87 (1912); 108 (1913); 164 (1915); *J. Biol. Chem.*, 6, 39 (1909); *Botan. Gaz.*, 50, 161 (1916); Schreiner, *8th Intern. Cong. Appl. Chem. Orig. Com.*, 15, 231 (1912); Skinner, *Ibid.*, 15, 253 (1912); *Plant World*, 18, 162, 168 (1915).

[3] J. Davidson, *J. Am. Soc. Agr.*, 7, 148–158, 221–238 (1915); F. W. Upson and A. R. Powell, *J. Ind. Eng. Chem.*, 7, 420–422 (1915).

[4] G. S. Fraps, Texas Agr. Expt. Sta., *Bull.* 174 (1915); W. J. Robbins, *Science*, 44, 894–895 (1916).

[5] J. J. Skinner, U. S. Dept. Agr., *Bull.* 164 (1915).

[6] S. L. Jodidi, Iowa Agr. Expt. Sta., *Res. Bull.* 9 (1912); E. C. Lathrop, *Soil Science*, 1, 509–532 (1916).

investigation on this line with the studies of the influence of chemical structure of some organic nitrogenous compounds upon the rate of their ammonification.

In this place the author wishes to express his hearty thanks to Dr. Oswald Schreiner and Dr. Elbert C. Lathrop, by whose kindness this experiment has been carried out in the laboratory of Soil Fertility Investigations, Bureau of Plant Industry, U. S. Department of Agriculture.

Experimental.

The soils used for the experiment were Caribou silt loam and Washburn loam from Maine, Superior clay from Wisconsin, Scottsburg silt loam from Indiana and soil from the Arlington farm, Virginia, collected by the Division of Soil Fertility Investigations. These soils were used in air-dried condition after having passed through a 1 mm. sieve. Leucine, tyrosine, acetanilide, benzanilide, acetamide and benzamide were selected as the chemicals to be used. The two first compounds were made in the laboratory of Soil Fertility Investigations by us. The other four compounds were purchased and used for the experiment after purification.

For the experiment about 300 cc. wide-mouth bottles were used. Ten cc. of water were added to 100 g. of soil to which had been added 100 mg. equivalents of nitrogen of each compound to be examined and well mixed. The amount of each compound equivalent to 100 mg. of nitrogen is as follows:

Leucine, 0.9372 g.; tyrosine, 1.2943 g.; acetanilide, 0.9650 g.; benzanilide, 1.4079 g.; acetamide, 0.4221 g.; benzamide, 0.8650 g.

The treated soils were kept in bottles. After incubation for certain intervals of time, as indicated in the following table, 10 g. of the mixture were transferred into Kjeldahl flasks and the ammonia was determined by the usual distillation method with magnesia. In all cases the amount of ammonia obtained by distillation of the control, that is, the soil without addition of any compound, was subtracted from the quantity of ammonia found on distillation of the treated soils after they had undergone the process of ammonification. Care was taken to know the loss of moisture by evaporation and it was found that no appreciable loss occurred during the experiment.

The results obtained are shown in the table.

Making due allowance for the rather wide variation in the amount of ammonia produced from the compound in different soils, we note from these results that on the whole acetamide was ammonified to the greatest extent of any of the compounds examined. The amount of ammonia formed from leucine was smaller than from acetamide, but greater than from tyrosine. The amount of ammonia from tyrosine was smaller than

TABLE SHOWING MG. OF AMMONIA NITROGEN PRODUCED FROM THE COMPOUNDS EQUIVALENT TO 100 MG. OF NITROGEN IN 100 G. OF SOILS.

Soils.	Leucine.	Tyrosine.	Acetanilide.	Benzanilide.	Acetamide.	Benzamide.
After Incubation for 2 Days.						
Arlington.....	7.77	3.12	Trace	Trace	13.25	Trace
Caribou.....	7.77	3.12	Trace	Trace	6.18	Trace
Indiana.....	8.32	3.34	Trace	Trace	9.28	Trace
Superior.....	15.53	7.23	Trace	Trace	12.55	1.55
Washburn.....	10.87	12.46	Trace	1.56	15.46	1.55
After Incubation for 7 Days.						
Arlington.....	46.59	24.93	Trace	Trace	86.57	Trace
Caribou.....	29.95	26.71	Trace	Trace	51.90	Trace
Indiana.....	34.17	15.58	Trace	Trace	77.30	1.55
Superior.....	40.38	40.51	1.55	1.56	86.57	6.21
Washburn.....	69.87	45.19	1.55	1.56	86.57	4.66
After Incubation for 12 Days.						
Arlington.....	71.44	50.08	Trace	Trace	86.57	Trace
Caribou.....	59.02	57.87	Trace	Trace	86.57	Trace
Indiana.....	57.13	30.61	Trace	1.56	92.30	3.10
Superior.....	65.23	54.43	6.21	6.20	74.20	10.86
Washburn.....	79.20	61.21	6.21	6.24	86.57	7.75
After Incubation for 16 Days.						
Arlington.....	77.66	61.21	Trace	Trace	86.57	3.10
Caribou.....	73.22	69.00	Trace	Trace	86.57	3.10
Indiana.....	71.44	38.40	3.10	3.10	95.52	4.66
Superior.....	77.66	57.65	9.32	9.36	71.11	15.52
Washburn.....	82.32	70.12	13.98	14.04	86.57	13.95
Average of highest amount of ammonia nitrogen produced from each compound and soil.....	76.46	59.28	5.28	5.30	88.36	8.07

that from leucine, however, much greater than from benzamide, acetanilide and benzanilide. The amount of ammonia from benzamide was greater than that from acetanilide or benzanilide while the ammonia from the latter two compounds was almost the same and the lowest among the compounds tested. The order of these compounds in respect to the rate of ammonification is, therefore, as follows:

Acetamide > Leucine > Tyrosine >

Benzamide > Acetanilide > Benzanilide.

The differences existing between these compounds as to the rate of ammonification are, since all conditions of the experiment like moisture, temperature, amount of soils, etc., were equal, to be attributed to the chemical structure of the compounds used. The following formulas show the chemical structure of these compounds:

Leucine, $(CH_3)_2CH.CH_2.CH.(NH_2)COOH$; tyrosine, $HOC_6H_4.CH_2.CH(NH_2)COOH$; acetanilide, $C_6H_5.NH.CO.CH_3$; benzanilide, $C_6H_5.NH.CO.C_6H_5$; acetamide, $CH_3.CO.NH_2$; benzamide, $C_6H_5.CO.NH_2$.

A glance at the structural formulas of these compounds shows that acetamide and leucine belong to the compounds of the fatty series, while the other four compounds are aromatic compounds. The nitrogen to be ammonified in all these compounds does not exist in the same form. In some of them it exists in the form of the amino group, while in others in the form of the imino group. Leucine, tyrosine, acetamide and benzamide contain the nitrogen in the form of the amino group. The amino group in the former two compounds is combined to the α-carbon atom while that of the latter two substances exists in amide form combined with a carbonyl group. In acetanilide and benzanilide, however, the nitrogen exists in the form of the imino group and combined directly with the benzene ring.

Taking the above results of ammonification and chemical structure of the compounds into consideration, we can conclude that in general fatty compounds seem to be more easily ammonified than aromatic compounds, owing to the influence of the benzene ring. Among the compounds of the aromatic series, the compounds of which nitrogen exists in the form of the imino group like acetanilide and benzanilide, seem to be more difficultly ammonified than the compounds in which nitrogen takes the form of the amino group, as in the case of tyrosine and benzamide. The nature of the other group does not seem to have any influence upon the rate of transportation of imino nitrogen into ammonia since it is shown by our results that acetanilide and benzanilide have been ammonified at almost the same rate.

As to the difference of behavior of amino acids and acid amides in respect to the rate of ammonification, our results do not lead to any definite conclusions since our comparison was not made with amino acids and acid amides of equal structure like glycocoll and acetamide, alanine and propionamide, phenylalanine and phenylpropionamide, etc. According to Jodidi,[1] however, the rate of transformation of amino acid and acid amide nitrogen into ammonia nitrogen is greatly influenced by the chemical structure of the compound so that amino acids and acid amides of equal structure yield about the same proportion of ammonia and *vice versa*.

Summary.

1. The rate of ammonification is greatly influenced by the chemical structure of the compound.

2. Fatty amino compounds seem to be ammonified much more easily than aromatic amino compounds.

3. Aromatic imino compounds seem to be much more difficultly ammonified than aromatic amino compounds.

4. The nature of the other group in the anilide molecule does not seem

[1] *Loc. cit.*

to have any influence upon the rate of transformation of imino nitrogen into ammonia nitrogen.

SAPPORO, JAPAN.

[CONTRIBUTION FROM THE BUREAU OF STANDARDS.]

GAS INTERFEROMETER CALIBRATION.[1]

BY JUNIUS DAVID EDWARDS.

Received August 8, 1917.

The Rayleigh-Zeiss gas interferometer has found numerous applications in high precision gas analysis. However, it requires preliminary calibration and its accuracy is limited by the accuracy of the calibration even though the precision of reading be considerably greater. This interferometer and its uses have been described in detail by Haber and Lowe,[2] L. Stuckert,[3] L. H. Adams,[4] and others.

This type of gas interferometer measures the difference in refractivity of two samples of gas contained in two gas chambers which in the laboratory type of apparatus are 100 cm. long. Light from an illuminated slit passes through both chambers, after which the two beams combine to produce interference fringes which are observed through an eye piece. The optical path of the two beams can be brought to equality by tilting a glass compensator plate which is placed in the path of one of the beams. If the temperature, pressure, or composition of the gas in one of the chambers is changed, the optical paths are different and the interference fringes are shifted. The fringes are brought back to their original position by tilting the compensator plate. The angle through which the compensator plate has been tilted, which is measured by means of a drum attached to the micrometer screw, is a measure of the change in refractive index of the two gases.

The customary method of calibration is to place a standard gas in one chamber and to determine the number of scale divisions the fringes are shifted when the second chamber contains the standard gas plus various known percentages of the gas for which the instrument is being calibrated; the gases in the two chambers are kept at the same temperature and pressure. The results are, of course, no more accurate than the method of analysis of the mixtures, although the precision of reading may be considerably greater.

L. H. Adams[5] has shown how the sensitivity of the interferometer (water or gas) can be calculated from certain dimensions and constants

[1] Published with the permission of the Director, National Bureau of Standards.

[2] Haber and Lowe, *Z. angew. Chem.*, **23**, 1393 (1910).

[3] Stuckert, *Z. elektrochem.*, **16**, 37 (1910).

[4] L. H. Adams, THIS JOURNAL, **37**, 1181 (1915).

[5] *Loc. cit.*